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Evaluating UV-B effects and EDU protection in cucumber leaves using fluorescence images and fluorescence emission spectra

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Summary

A newly developed laboratory fluorescence imaging system was used to obtain fluorescence images (FImage) of freshly excised cucumber (*Cucumis sativus* L.) leaves in spectral bands centered in the blue (F450), green (F550), red (F680), and far-red (F730) spectral regions that resulted from a broad-band (300–400 nm) excitation source centered at 360 nm. Means of relative fluorescence intensities (RFI) from these spectral fluorescence images were compared with spectral fluorescence emission data obtained from excitation wavelengths at 280 nm (280EX, 300–550 nm) and 380 nm (380EX, 400–800 nm) of dimethyl sulfoxide (DMSO) extracts from these leaves. All three fluorescence data types (FImage, 280EX, 380EX) were used to assess ultraviolet-B (UV-B, 280–320 nm) induced physiological changes and the possible use of N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N′-phenylurea (EDU or ethylenediurea) as a chemical protectant against UV-B damage. Plants exhibited well known foliar growth and pigment responses to UV-B exposure (e.g., increased UV-B absorbing compounds and decreased leaf area, chlorophyll *a* content; and and lower chlorophyll *a/b* and chlorophyll/carotenoid pigment ratios). Since EDU alone had no effect on foliar variables, there was no evidence that EDU afforded protection against UV-B. Instead, EDU augmented some UV-B effects when provided in conjunction with UV-B irradiation (e.g., reductions in the chlorophyll/carotenoid ratio, total photosynthetic pigments, and chlorophyll *b* content).

Relative fluorescence intensities (RFI) in the longer visible wavelengths (green, red, and far-red) were uncorrelated for comparisons between the FImage and 380EX data sets. However, blue and green RFI were significantly correlated ($0.8 > r > 0.6$; $P \leq 0.002$) for comparisons between FImage and 280EX data sets. UV-B treatment caused an increase in blue RFI (e.g., F450) in both images and 280EX measurements. One explanation is that the UV-B excitation of both 280EX and FImage stimulates processes that produce excess blue fluorescence. The molecules that produce the excess blue fluorescence in both the 280EX and the Fimage data are different electron transfer agents that operate in parallel. For FImage, the UV excitation penetrates leaf surface layers to stimulate fluorescence from compounds in mesophyll and epidermal tissues (as occurs for the extracts of leaf discs),

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whereas emissions captured at longer, less energetic wavelengths, were primarily from the epidermal layer. UV-B irradiated leaves showed much greater heteorgeneity of RFI in both the green ($F550_{FImag}$) and the red ($F680_{FImag}$) bands than unirradiated leaves; this was true irrespective of EDU treatment.

Although qualitative responses in individual bands differed between FImage and 380EX data, similar results were obtained in the detection of UV-B induced effects when the red/green and blue/far-red fluorescence ratios of these data were compared. The red/green ratio (either $F680/F550_{FImage}$ or $F675/F525_{380EX}$) was lower for UV-B exposed plants in both images and 380EX data. UV-B exposure also significantly enhanced the blue/far-red ratio of images ($F450/F740_{FImage}$) and the comparable 380EX ratio ($F450/F730_{380EX}$) for the combined UV-B/EDU group. The far-red/red ratios were not useful in separating treatment effects in images or 380EX. Although comparable ratios were not available in 280EX data, the UV/blue ratio ($F315/F420_{280EX}$) was substantially reduced by UV-B exposure and was inversely related to total photosynthetic pigment content. These findings suggest that the red/green ratio (FImage, 380EX) and the UV/blue ratio (280EX) may be as useful as the blue/far-red ratio (380EX) reported previously in detection of UV-B stress. Furthermore, the results support the validity of the imaging technique as a non-destructive diagnostic tool for assessing UV-B stress damage in plants.

Key words: Blue, green, red, and far-red fluorescence – cucumber (*Cucumis sativus* L.) – EDU – fluorescence imaging – fluorescence ratios red/green, blue/far-red, UV/blue, far-red/red – plant stress – UV-B

Abbreviations: DMSO dimethyl sulfoxide. – EDU (N-[2-(2-oxo-1-imidiazolidinyl) ethyl]-N′-phenylurea) or ethylene diurea. – F fluorescence. – F450 blue fluorescence. – F550 green fluorescence. – F680 red fluorescence. – F740 far-red fluorescence. – FImage fluorescence image. – FIS fluorescence imaging system. – HPS high pressure sodium. – LPS low pressure sodium. – MH metal halide. – MED minimum erythemal dose. – PAR photosynthetically active radiation (400–700 nm). – PS I Photosystem I. – PS II Photosystem II. – RFI relative fluorescence emission intensity. – RLA Relative leaf area. – UV-A ultraviolet A radiation (320–400 nm). – UV-B ultraviolet B radiation (280–320 nm). – $UV\text{-}B_{BE}$ biologically effective UV-B radiation. – 280EX fluorescence emission spectra obtained from 300–530 nm, produced by excitation at 280 nm. – 380EX fluorescence emission spectra obtained from 400–800 nm, produced by excitation at 380 nm

Introduction

Plants in their natural environment are often exposed to a variety of natural and anthropogenic stress conditions (Lichtenthaler 1990, 1996, Lang et al. 1991, 1996, Lichtenthaler and Rinderle 1988). An important environmental factor contributing to plant stress is ultraviolet-B (UV-B) radiation (280–320 nm), which at higher than natural levels can adversely affect photosynthesis and other physiological processes (Bornman et al. 1994, Strid et al. 1990, Tevini 1993, 1994). In addition to stratospheric ozone depletion by chlorofluorocarbons over Earth's polar regions (Blumthaler and Ambach 1988), ozone depletion and consequent increases in UV irradiation documented at mid-latitudes (Herman et al. 1996) could also potentially affect crop heartlands.

Under normal conditions, most of the solar energy absorbed by leaf pigments (chlorophylls and carotenoids) is used for photosynthesis and a small amount is released as infrared radiation (heat) and as red chlorophyll fluorescence. Under stress conditions, photosynthesis declines while heat emission and chlorophyll fluorescence increase considerably (Lichtenthaler 1996). The use of chlorophyll fluorescence measurements for detection of physiological stresses in plants has been well documented (Larsson et al. 1998, Lichtenthaler 1990, 1996, 1997, Lichtenthaler and Rinderle 1988, Snell and van Kooten 1990). Variable fluorescence measurements have been used to assess the physiological effects of UV-B radiation on cucumber (Middleton et al. 1996), snap bean (Agrawal et al. 1991), sunflower (Tevini and Pfister 1985, Tevini et al. 1988), and other species. The blue/far-red fluorescence ratio obtained from emissions of leaf extracts was previously identified as an indicator of general plant stress (Stober et al. 1994, Lang et al. 1994) and was subsequently successfully applied to the demonstration of UV-B induced stress (Subhash et al. 1995, Middleton et al. 1996). Middleton et al. (1996) also found that increases in the blue/far-red ratio induced by UV-B radiation were similar, whether obtained from

leaf extracts or whole leaves, although the latter were more variable.

Plant tolerance to environmental stressors may be enhanced by applying plant growth regulators and antioxidants (Lee et al. 1992). One chemical compound that has been found to induce cellular defense against ozone damage in a number of plant species is ethylenediurea (EDU : N-[2-(2-oxo-1-imidiazolidinyl) ethyl]-N′-phenylurea, Lee and Bennett 1985). EDU has been shown to provide protection against foliar injury from ozone if applied as a foliar spray or through soil application (Carnahan et al. 1978, Cathey and Heggestad 1982, Kosta-Rick and Manning 1993, Miller et al. 1994). In a few studies, high concentrations of EDU have been reported as causing visible foliar injury and decreasing crop yield (Bennett et al. 1981, Kosta-Rick and Manning 1993, Miller et al. 1994). The possible protective effects of EDU against UV-B radiation are unknown.

Recent development of a fluorescence imaging system affords a tool for securing rapid, non-destructive detection of stress effects in whole, freshly excised, intact leaves. In addition to the desirability of having whole leaf analysis capability, this imaging approach is pursued because of the potential to identify stress symptoms before the onset of visible injury (Kim et al. 1996, Lang et al. 1994, 1996, Lichtenthaler 1996, 1997). Sandhu et al. (1997) previously reported on the effectiveness of EDU to ameliorate UV damage. The present study mainly evaluates the usefulness of fluorescence emission spectra and fluorescence images for UV-B stress detection in a cultivar of cucumber previously found sensitive to UV-B radiation (Krizek 1978, Adamse and Britz 1992, Krizek et al. 1994, Middleton et al. 1996). Measurements of steady state fluorescence emission spectra of DMSO leaf extracts and fluorescence imagery of whole leaves were the primary tools used to assess treatment effects on plant growth and pigment production.

Materials and Methods

Plant culture and treatments

Cucumber (*Cucumis sativus* L.) cv. ‹Poinsett› was grown in 12.7-cm diameter plastic pots containing a peat-vermiculite mix (Jiffy Mix, Jiffy Products of America, West Chicago, IL, USA), and fertilized daily with a complete nutrient solution, as described by Silvius et al. (1978). After four days, seedlings were thinned to one plant per pot. Plants were initially grown in a growth chamber containing high pressure sodium (HPS) and metal halide (MH) lamps (HPS/MH) for 10 days under the following conditions: day/night temperature, 27 °C; 50 % relative humidity; carbon dioxide (CO_2), 450 μmol mol^{-1}; and photosynthetically active radiation (PAR, 400–700 nm) of 840 μmol m^{-2} s^{-1} for 12 h between 0730 and 1930, and 270 μmol m^{-2} s^{-1} for 1 hr each at the beginning and end of the photoperiod (0630–0730 and 1930–2030), provided by an equal mix of 400 W HPS and 400 W MH lamps. On day 9, plants were given a soil drench of EDU at either 0 or 500 μmol mol^{-1}. Since the average mass of the dry Jiffy Mix was 200 g, the EDU concentration can also be expressed as either 0 or 250 μg g^{-1} of dry mix.

On day 11, when the third leaf was unfolding (approximately 1 cm^2 in area), the plants were transferred for UV treatment to a separate plant growth chamber containing 180 W low pressure sodium (LPS) lamps (Philips North America, Bloomfield, NJ, USA), to provide background PAR, and supplemental UV lamps. The UV exposure chamber was maintained at the same environmental conditions (photoperiod, temperature, relative humidity, PAR, and CO_2) as described for the HPS/MH growth chamber. The LPS chamber was divided into two compartments by a vertical sheet of UV-B absorbing polyester film. On each side, UV radiation was provided for 8 h midway through the 14 h photoperiod by UV-B 313 fluorescent sunlamps (Q-Panel Lab Products, Cleveland, OH, USA) mounted horizontally over the plants. The plants were exposed for 2 days at 0.2 or 18 kJ m^{-2} d^{-1} of biologically effective UV-B ($UV\text{-}B_{BE}$) radiation, respectively, normalized to unity at 300 nm (Caldwell 1971). UV-B lamps were wrapped with collars of either polyester (0.13 mm thick) or cellulose acetate (0.08 mm) to exclude or transmit UV-B. UV-B irradiance at plant level was monitored and adjusted by means of a portable UV radiometer [Minimum Erythemal Dose (MED) Meter, Solar Light Co., Philadelphia, PA, USA], calibrated with a UV spectroradiometer (Model 752, Optronic Laboratories, Inc., Orlando, FL, USA). Following UV-B irradiation, plants were returned to the HPS/MH growth chamber.

Growth measurements and leaf injury

Measurements of relative leaf area (RLA, product of leaf length and leaf width) of the third leaf were made on day 11 (2 days after EDU application and prior to UV-B irradiation) and at 34 and 72 h thereafter to determine the effects of EDU and UV-B irradiation on leaf expansion. On day 14, visual damage of the third leaf was evaluated and absolute leaf area measurements were made (LICOR, Model LI–3000, Lincoln, NE, USA).

Fluorescence spectral images

After scoring the plants for UV-B injury on day 14, the third leaf was excised from each plant. A fluorescence imaging system (FIS) was used to acquire two-dimensional steady-state fluorescence images of whole leaves in four spectral bands centered at wavelengths in the blue (450 nm), green (550 nm), red (680 nm), and far-red (740 nm) regions, designated as $F450_{FImage}$, $F550_{FImage}$, $F680_{FImage}$, and $F740_{FImage}$, respectively (Fig. 1). The band width at half peak maximum was 25 nm in the blue and green regions and 10 nm in the red and far-red regions (Kim et al. 1997). A broad-band (300–400 nm) UV excitation source centered on 360 nm (UV-A fluorescent lamps, Model EA-180/12, Spectroline Inc., USA) was filtered with Schott UG-1 glass to eliminate any radiation over 400 nm. In addition to the excitation source, the FIS consists of a thermo-electrically cooled digital camera and optics [charge coupled device (CCD) camera, Lynxx-2, Spectra Source Instruments, Westlake Village, CA, USA], and a desktop computer interface for data storage and instrument control.

Lamina of freshly excised whole leaves were placed horizontally on a platform (painted non-fluorescent flat black) with adaxial surfaces facing upward toward the CCD camera. Each image was comprised of four leaves, one from each treatment, arranged as shown in Figure 2. Spectral means of RFI for major portions of the adaxial leaf surface were determined from the images.

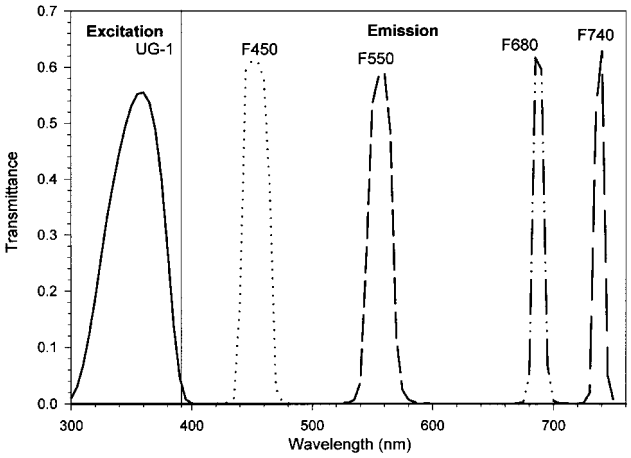

Figure 1. The relative transmittance of the Schott UG-1 glass filter used for fluorescence excitation and the four emission bands used to obtain fluorescence images. Peak bandwidths are centered on 450 (blue), 550 (green), 680 (red) and 740 (far-red) nm, and are designated as $F450_{FImage}$, $F550_{FImage}$, $F680_{FImage}$, and $F740_{FImage}$.

Fluorescence emission spectra

Immediately after fluorescence images were acquired, two leaf disks (1 cm diameter each) were removed from the distal portion of the third leaf of each plant (near the leaf edge) for determination of fluorescence emission spectra and extraction of photosynthetic pigments, as described by Middleton et al. (1996). The disks were extracted in 4 mL dimethyl sulfoxide (DMSO) and kept for 24 h in the dark. To prevent saturation of emission values, 20 µL of each DMSO leaf extract was diluted with DMSO and the total volume was brought to 4 mL in a quartz cuvette. A spectrofluorometer (Fluorolog II, SPEX Industries, Inc., Edison, NJ, USA) with two double monochrometers was used to collect steady-state fluorescence emission spectra from the leaf extracts.

Two emission spectra per sample were obtained with excitation wavelengths at 280 and 380 nm (designated as 280EX and 380EX, respectively). For 280EX, the emission wavelength range was 300 to 530 nm; for 380EX, the emission wavelength range was 400 to 800 nm. Emission spectra were obtained by fixing the excitation wavelength and recording the emission intensity while stepping from shorter to longer wavelengths in the spectral range. RFI from 380EX spectra were evaluated at these wavelengths: 420, 435, 450, 525, 675, and 730 nm, and are designated as $F420_{380EX}$, $F435_{380EX}$, $F450_{380EX}$, $F525_{380EX}$, $F675_{380EX}$, and $F730_{380EX}$, respectively. RFI from 280EX spectra were evaluated at these wavelengths: 315, 335, 420, 450, 475, and 525 nm, and are designated as $F315_{280EX}$, $F335_{280EX}$, $F420_{280EX}$, $F450_{280EX}$, $F475_{280EX}$, and $F525_{280EX}$, respectively.

Photosynthetic pigments and UV-B absorbing compounds

Samples of the pigment extracts were placed in a standard quartz cuvette and analyzed in a computerized dual beam spectrophotometer (Lambda 3B, UV/VIS, Perkin-Elmer, Norwalk, CN, USA), with a 1 nm resolution. The absorption spectra were scanned from 400 to 750 nm. Concentrations of photosynthetic pigments were calculated using equations described by Chappelle et al. (1992) and expressed on a per area basis ($\mu g\ cm^{-2}$).

An additional four leaf discs (1 cm diameter each) were removed from the distal portion of the designated leaf, and UV-B absorbing compounds were extracted in ethanol acidified with glacial acetic acid (ethanol : acetic acid; v : v, 99 : 1). The discs were boiled gently for 10 min in a water bath at 80 °C, and absorbances were read at 270, 300, and 330 nm using a UV-Visible Recording Spectrophotometer (UV-160A, Shimadzu, Columbia, MD, USA).

Statistical analyses

The experimental design was a 2×2 factorial with two levels of UV-B radiation and two levels of EDU with replication in time; there were five samples per treatment for each experiment. The four resultant treatments were: 1) Control (no UV, no EDU); 2) EDU treatment (−UV-B/+EDU); 3) UV-B treatment (+UV-B/−EDU); and 4) combined +UV-B/+EDU treatment (+UV-B/+EDU). The data were analyzed using Systat 5.01 (SYSTAT, Inc., Evanston, IL, USA) for spectral emission data and photosynthetic pigments and PC SAS 6.11 (SAS Institute, Inc., Cary, NC, USA) for UV absorbing compounds and growth responses. The effects of EDU and UV-B treatments and their interactions were evaluated for growth responses, pigment content, and fluorescence emissions. Results are reported below for means and standard errors of the mean ($\bar{x} \pm SE$) of select groups.

Results

Growth measurements and leaf injury

Relative leaf area (RLA) and rate of leaf expansion after 34 and 72 h were significantly reduced by UV-B exposure (Table 1). Absolute leaf area of the third leaf at the end of the experiment was 37 or 47 % less ($P \leq 0.001$), respectively, in UV-B versus non-UV treated plants, regardless of EDU (Table 2). In cucumber plants exposed to UV-B radiation, the third leaf also showed a prominent wrinkling of the lamina and typical chlorotic spots. RLA, rate of leaf expansion, and absolute leaf area were all unaffected by EDU (either prior to, or after UV-B exposure) (Tables 1, 2), and there was no apparent foliar injury caused by EDU.

Photosynthetic pigments and UV-B absorbing compounds

Pigment concentration data are summarized in Table 2. UV-B radiation induced an 8–10 % decrease in concentration of chlorophyll *a* and total chlorophyll, regardless of EDU. Chlorophyll *b* concentration decreases only occurred in plants receiving the combined UV-B/EDU-treatment. UV-B treatment significantly reduced ($P \leq 0.002$) the chlorophyll *a/b* ratio, but the chlorophyll/carotenoid ratio was only reduced in the combined UV-B/EDU treatment. UV-B treated plants (in the absence of EDU) showed a significant increase in absorbance at 300 nm, when expressed on a leaf area basis. A significant EDU × UV-B interaction was observed at both 300 nm (Table

Table 1. Relative leaf area (RLA) in the third emerging leaf of 'Poinsett' cucumber after 0, 34, and 72 h of UV-B treatment (0.2 and 18 kJ m^{-2} d^{-1}, −UV-B and + UV-B respectively), changes in RLA between 0–34 h and 34–72 h, showing means and standard error of the mean, and ANOVA summary showing F value and level of significance. EDU was applied 48 h prior to UV-B iradiation at a concentration of 0 or 500 mol mol^{-1}.

EDU conc.	UV-B trt.	RLA (cm^2)*			Change in RLA (cm^2)*	
		0 h	34 h	72 h	0–34 h	34–72 h
0	–	4.8±0.3 a	19.7±1.5 a	96.3±4.0 a	14.9±1.3 a	76.6±2.7 a
0	+	5.2±1.1 a	12.2±1.5 b	48.8±4.9 b	7.0±1.1 b	36.7±3.4 b
500	–	4.2±0.5 a	17.0±1.7 a	88.5±5.5 a	12.8±1.3 a	71.5±4.6 a
500	+	4.6±0.4 a	12.7±1.4 b	50.9±4.1 b	8.1±1.1 b	38.3±2.7 a
Source of Variation		ANOVA Summary (F values)**				
EDU		0.9^{NS}	0.8^{NS}	0.6^{NS}	0.3^{NS}	0.3^{NS}
UV-B		0.3^{NS}	24.6***	22.4***	44.6***	42.3***
UV-B×EDU		0.0^{NS}	1.8^{NS}	1.7^{NS}	2.9^{NS}	1.2^{NS}

* Values differing by a letter are significantly different at $P \leq 0.05$; NS = non-significant.

** Statistically significant at $P \leq 0.001$; NS = non-significant.

Table 2. Effect of experimental treatments on leaf size and leaf pigments at end of experiment. EDU treatment was either 0 or 500 μmol mol^{-1}, and UV-B treatment was either 0.2 or 18 kJ m^{-2} d^{-1}.

Variable	Experimental Treatment			
	Control	EDU	UV-B	UV-B/EDU
Absolute Leaf Area (cm^2)	79.80±3.60 a	73.50±4.90 a	42.30±3.10 b	46.00±4.00 b
Chlorophyll *a* μg cm^{-2}	28.15±1.82 a	28.45±1.54 a	25.58±0.62 b	23.38±1.49 b
Chlorophyll *b* μg cm^{-2}	12.91±0.76 a	12.70±0.57 a	12.24±0.35 a	10.96±0.64 b
Chlorophyll *a/b* ratio	2.18±0.02 a	2.23±0.03 a	2.09±0.03 b	2.13±0.03 a, b
Chlorophyll/Carotenoid ratio	8.06±0.35 a	8.02±0.27 a	7.04±0.48 a, b	6.65±0.42 b
Total Photosynthetic Pigment (μg cm^{-2})	46.23±2.92 a	46.37±2.45 a	43.39±1.05 a, b	39.65±2.41 b
$A_{300\ nm}$ (Abs cm^{-2})*	2.74±0.09 a	2.88±0.06 a	3.17±0.11 b	2.92±0.00 a

* A significant ($P \leq 0.01$) UV-B×EDU interaction was observed.

2) and at 330 nm (data not shown). Neither UV-B nor EDU had any effect on carotenoid concentration (data not shown).

Although UV-B absorbing compounds often increase after UV-B exposure, a documented plant defense mechanism (Mirecki and Teramura 1984), no direct link to fluorescence measurements was observed. The significant EDU×UV-B interaction (Table 2) may have confounded attempts to link UV-B absorbing compounds to fluorescence measurements.

Fluorescence measurements

Similar magnitudes in the RFI intensities were obtained for the 280EX and 380EX spectra, with values between ~1 and 43×10^4 (Table 3). Compared with 380EX, 280EX emissions were almost twice as great in the blue and more than three times as great in the green region. The relative values per band obtained by the FIS for the FImage cannot be directly compared with those acquired by the spectrofluorometer, due to different gain settings and instrument characteristics. However, substantial differences in spectral responses among the measurement types were expressed in the fluorescence ratios (Table 3).

UV-B irradiation had a pronounced effect on the spatial distribution of the fluorescence emissions; this is illustrated in the green band ($F550_{FImage}$) and in the red band ($F680_{FImage}$) (Fig. 2). In addition, FImages of UV-B irradiated leaves showed much greater heterogeneity in pixel intensity (RFI) than those of unirradiated leaves, (see dotted horizontal line in Fig. 2 b). This was true for both EDU and non-EDU treated plants (see dotted vertical lines in Fig. 2 a and b) and for both the green ($F550_{FImage}$) and red ($F680_{FImage}$) bands. The large variation in RFI observed in the interveinal regions in the red band may indicate heterogenous states in the photosynthetic apparatus that could be due to localized differences in chlorophyll concentration and photosynthetic rate within the leaf fol-

Table 3. Experiment-wide means and standard errors ($\bar{x} \pm SE$) for steady state fluorescence emissions acquired in four spectral bands (FImages) by the Fluorescence Image System (FIS), and at select emission wavelengths acquired by the Fluorolog II spectrofluorometer that were produced from excitation wavelengths of 280 nm (280 EX) and 380 nm (380 EX). Values are also given for select spectral ratios.

Measurement Type	Spectral Region	Waveband Center (nm)	Symbol	RFI $\bar{x} \pm SE$
I. Individual Bands				
FImage	blue	450	$F450_{FImage}$	43.01± 1.12
	green	550	$F550_{FImage}$	37.50± 1.23
	red	680	$F680_{FImage}$	660.67±18.14
	far-red	740	$F740_{FImage}$	923.45±21.31
280 EX	UV-B	315	$F315_{280\,EX}$	$8.08 \pm 0.12 \times 10^4$
	UV-A	335	$F335_{280\,EX}$	$16.10 \pm 0.50 \times 10^4$
	blue	420	$F420_{280\,EX}$	$3.52 \pm 0.11 \times 10^4$
	blue	450	$F450_{280\,EX}$	$2.71 \pm 0.09 \times 10^4$
	blue	475	$F475_{280\,EX}$	$2.34 \pm 0.07 \times 10^4$
	green	525	$F525_{280\,EX}$	$3.29 \pm 0.10 \times 10^4$
380 EX	blue	420	$F420_{380\,EX}$	$1.44 \pm 0.04 \times 10^4$
	blue	435	$F435_{380\,EX}$	$2.23 \pm 0.05 \times 10^4$
	blue	450	$F450_{380\,EX}$	$1.74 \pm 0.05 \times 10^4$
	green	525	$F525_{380\,EX}$	$0.88 \pm 0.02 \times 10^4$
	red	675	$F675_{380\,EX}$	$42.78 \pm 1.28 \times 10^4$
	far-red	730	$F730_{380\,EX}$	$6.94 \pm 0.23 \times 10^4$
II. Band Ratios				
FImage	red/green	680/550	$F680/F550_{FImage}$	20.980±1.470
	blue/far-red	450/740	$F450/F740_{FImage}$	0.041±0.002
	blue/green	450/550	$F450/F550_{FImage}$	1.153±0.015
	far-red/red	740/680	$F740/F680_{FImage}$	1.356±0.016
280 EX	UV-B/blue	315/420	$F315/F420_{280\,EX}$	2.600±0.067
	blue/green	450/525	$F450/F525_{280\,EX}$	0.845±0.031
	UV-A/blue	335/450	$F335/F450_{280\,EX}$	6.102±0.244
380 EX	red/green	675/525	$F675/F525_{380\,EX}$	54.710±2.270
	blue/far-red	450/730	$F450/F730_{380\,EX}$	0.220±0.009
	blue/green	450/525	$F450/F525_{380\,EX}$	1.969±0.023
	far-red/red	730/675	$F730/F675_{380\,EX}$	0.164±0.001

lowing UV-B exposure. These plots of variations in RFI are significant since they reveal information that would otherwise be masked by examining only mean values for RFI. The bright tips of the leaves are an artifact, and may reflect an accumulation of substances that fluoresce when excited by UV-A radiation. The margins of cucumber leaves are typically lighter green in color even in the absence of UV radiation.

The spatial differences in fluorescence emission in cucumber leaves following UV-B exposure are in contrast to findings that we have obtained in soybean (D. T. Krizek, M. S. Kim, E. M. Middleton, and R. K. Sandhu, unpublished results, 1996). In soybean, leaves of UV-B irradiated plants showed a uniform decrease in RFI when excited at 360 nm. These findings suggest that differences in flavonoids and other UV-B absorbing compounds may be partly responsible.

Spectral correlations

The RFI measured at red and far-red wavelengths were highly correlated ($r \geq 0.95$) *within* each of the FImage and the 380EX data sets (Table 4, Section I). Also, RFI measured at blue and green wavelengths were highly correlated within both the FImage ($r = 0.93$) data and the 380EX ($r = 0.90$) spectra. This indicates that red and far-red RFI provide similar information, as do blue and green RFI, within each of the FImage and 380EX data sets.

However, neither the red, the far-red, nor the green RFI were correlated *between* FImage and 380EX data sets (Table 4, Section II). For example, the red fluorescence of images ($F680_{FImage}$) of whole leaf adaxial surfaces was unrelated to comparable red 380EX fluorescence ($F675_{380EX}$) of leaf tissue

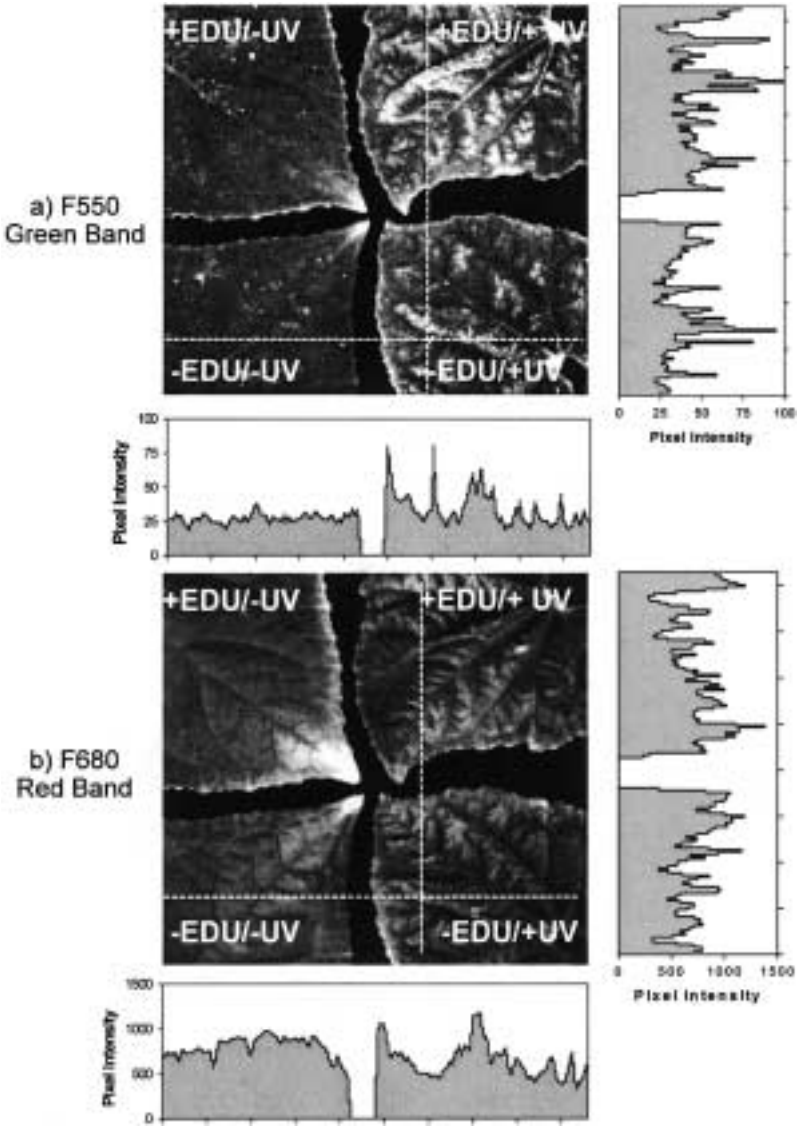

Figure 2. Typical fluorescence images of freshly detached leaves of ‹Poinsett› cucumber in the a) F550 (green, $F550_{FImage}$) and b) the F680 (red, $F680_{FImage}$) bands. Images were obtained on four leaves, each leaf representing one of the four experimental treatments. In each image, the Control (−UV-B/−EDU) is in the lower left corner. Other treatments are arranged clockwise: EDU treatment (−UV-B/+EDU), combined UV-B/EDU treatment (+UV-B/+EDU), and UV-B treatment (+UV-B/−EDU). A comparison of the right and left sets of each image illustrates the «+UV-B» vs. the «−UV-B» effect and a comparison of the top and bottom sets of images illustrates the «+EDU» vs. the «−EDU» effect. Note differences in relative fluorescence intensity (RFI) between the veinal and interveinal regions, and the high degree of heterogeneity of pixel intensity in UV-B irradiated leaves (see vertical dotted lines), irrespective of EDU treatment.

extracts. A similar lack of correlation was observed for the far-red bands, $F740_{FImage}$ vs. $F730_{380EX}$, and the green bands, $F550_{FImage}$ vs. $F525_{380EX}$. This indicates that red, far-red, and green RFI measured by FImage and 380EX conveyed altogether different information about leaf characteristics. Only in the blue spectrum was similarity between RFI of FImage and 380EX measurements apparent: RFI for $F450_{FImage}$ and $F450_{380EX}$ were correlated ($r = 0.54$; Table 4), indicating overlap in information content between these two measurements.

For 280EX data, only the green and blue RFI could be evaluated relative to comparability with FImage and 380EX measurements (red, far-red RFI were not acquired from excitation at 280 nm, shown in Fig. 1). RFI for FImage and 280EX were correlated in both the green ($r = 0.61$) and blue ($r = 0.79$) spectral regions (Table 4, Fig. 3 A, B), although RFI were unrelated in the comparable green band of FImage and 380EX. Consistent with this finding, RFI from extracts for 380EX and 280EX were uncorrelated in either the green or blue bands (Table 3, Fig. 3 A, B). Within the 280EX spectra, green and blue RFIs were correlated ($r = 0.77$), although not as strongly as was shown above for FImage and 380EX data (Table 4). Together, these results indicate that all measurement types (FImage, 280EX, and 380EX) measured similar leaf characteristics in the blue spectrum, and that similar leaf characteristics were captured in the green spectra by FImage and 280EX. RFI in the green and longer wavelength spectral regions of 380EX data were associated with different leaf tissue processes.

Experimental treatments

RFI differences associated with experimental treatments were most apparent in the blue spectrum for the FImage and the

Table 4. Correlations among spectral variables acquired using three differnt fluorescence measurements: fluorescence imaging of whole leaves (Image) and fluorescence of leaf extracts at two excitation wavelengths (280 and 380 nm).

Fluorescence Measurements Image	380EX	280EX	Correlation (r)	Significance P≤
I. Spectral Correlations Within Data Sets:				
A. red vs. far-red				
1. FI680 vs. FI740			0.95	0.000
2.	F675 vs. F730		0.96	0.000
B. blue vs. green				
1. FI450 vs. FI550			0.93	0.000
2.	F450 vs. F525		0.90	0.000
3.		F450 vs. F525	0.77	0.000
II. Spectral Correlations Across Data Sets:				
A. red				
FI680 vs.	F675		NS	
B. far-red				
FI740 vs.	F730		NS	
C. green				
1. FI550 vs.		F525	0.61*	0.002
2. FI550 vs.	F525		NS	
3.	F525 vs.	F525	NS	
D. blue				
1. FI450 vs.		F450	0.79*	0.000
2. FI450 vs.	F450		0.54*	0.010
3.	F450 vs.	F450	NS	

* 1 outlier removed from the analysis.

280EX (Fig. 4A, B) but not for the 380EX (Fig. 4C). $F450_{FImage}$ provided the greatest separation of treatments, with a clearly augmented response for the two UV-B treatments (Fig. 4A). Foliar $F450_{FImage}$ was highest from plants that received the combined UV-B/EDU treatment (Fig. 4A). In 280EX spectra, an enhanced UV-induced fluorescence ($P \leq 0.000$, $r^2 = 0.46$) was also expressed at $F450_{280EX}$ (Fig. 4B). In the 380EX data, however, apparently higher values for $F450_{380EX}$ were non-significant (Fig. 4C).

When RFI was averaged over the major portion of the adaxial leaf surface, no treatment differences were apparent in the green, red, or far-red FImage bands (data not shown). In 380EX spectra, significant UV-B effects were detected at these additional wavelengths: increased RFI for $F435_{380EX}$ and $F525_{380EX}$ ($P = 0.040$ and $P = 0.021$, respectively); decreased RFI for $F675_{380EX}$ ($P = 0.044$) and $F730_{380EX}$ ($P = 0.039$). No EDU effects on RFI or spectral ratios were observed for 380EX emission spectra. The only significant EDU effect for 280EX was observed in increased RFI for $F315_{280EX}$ ($P = 0.018$) in the presence of UV-B.

Success in separating treatments was achieved with fluorescence ratios in all data types (FImage, 380EX, and 280EX). In both the FImage and 380EX data sets, values significantly higher than controls were obtained for the combined UV-B/EDU treatment with blue/far-red fluorescence ratios (Fig. 5A, B): $F450/F740_{FImage}$ and $F450/F730_{380EX}$, respectively. The blue/green ratio of FImage was also significantly reduced ($r^2 = 0.37$) in the combined UV-B/EDU treatment, but treatment effects were non-significant in the comparable blue/green ratios from 280EX and 380EX.

UV-B effects were also clearly expressed in reductions of the red/green fluorescence ratios of both FImage ($P = 0.05$, $r^2 = 0.59$) and 380EX ($P = 0.002$, $r^2 = 27$) data; these red/green ratios are $F680/F550_{FImage}$ and $F675/F525_{380EX}$, respectively (Fig. 6). In 280EX spectra, UV-B effects were strongly exhibited in reductions ($P \leq 0.000$, $r^2 = 0.60$) of a UV/blue fluorescence ratio, $F315/F420_{280EX}$ (Fig. 7A). This $F315/F420_{280EX}$ ratio was inversely proportional ($r^2 = 0.45$) to total photosynthetic pigment content (Fig. 7B), with separate curves necessary for the UV-B and non UV-B treatments (UV-B < no UV-B).

No treatment effects were detected in the far-red/red fluorescence ratio $F730/F675_{380EX}$. The far-red/red ratio, $F740/F680_{FImage}$, was enhanced, however, in the combined UV-B/EDU group in images. Neither far-red/red ratio could be related to chlorophyll content.

Discussion

Our study illustrates the feasibility of using a high resolution fluorescence imaging system (FIS) for non-destructive analysis of UV-B effects and fluorescence emission spectral data

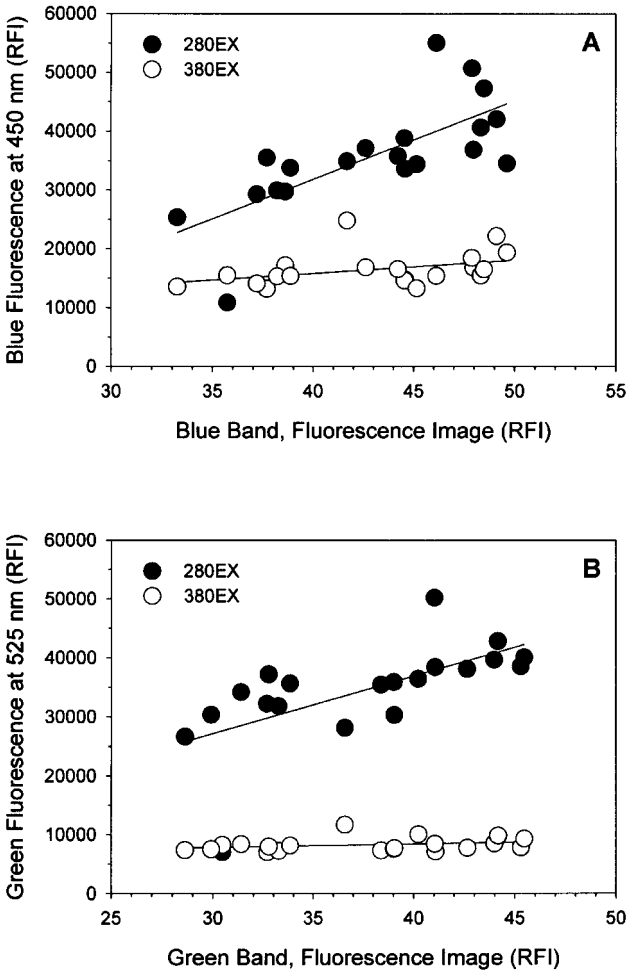

Figure 3. The correlations of fluorescence image means for adaxial whole leaf surfaces to comparable fluorescence of leaf extracts of these leaves are shown. The fluorescence measurements obtained from extracts were produced by excitation wavelengths in the UV-B at 280 nm (280EX) and in the UV-A at 380 nm (380EX). Results are presented for: A) the blue band centered at 450 nm (r_{280EX} = 0.79, $P \leq 0.000$ with one outlier shown eliminated from statistical analysis); and B) the green band centered at 550 or 525 nm (r_{280EX} = 0.61, P = 0.002 with one outlier shown eliminated from statistical analysis).

from DMSO extracts to get a more complete analysis of foliar stress damage. These two fluorescence measurement techniques are complementary with one another. The FIS was used to obtain fluorescence patterns across the entire leaf surface at selected wavelengths (blue, green, red, and far-red), whereas emission spectra obtained by excitation at 280 and 380 nm provided averaged fluorescence values over a large and continuous spectral range of subsamples (e.g., leaf discs). Our UV-B results are consistent with findings reported by Middleton et al. (1996). These investigators obtained good agreement with a spectrofluorometer in distinguishing UV-B effects in fresh leaves and 380EX of leaf extracts, although whole leaf responses were too variable to determine significant differences. In our study, only FImage measurements were taken on fresh leaves to determine UV-B radiation effects.

The success of the blue/far-red (F450/F730) fluorescence ratio as a reliable indicator of UV-B damage in our study

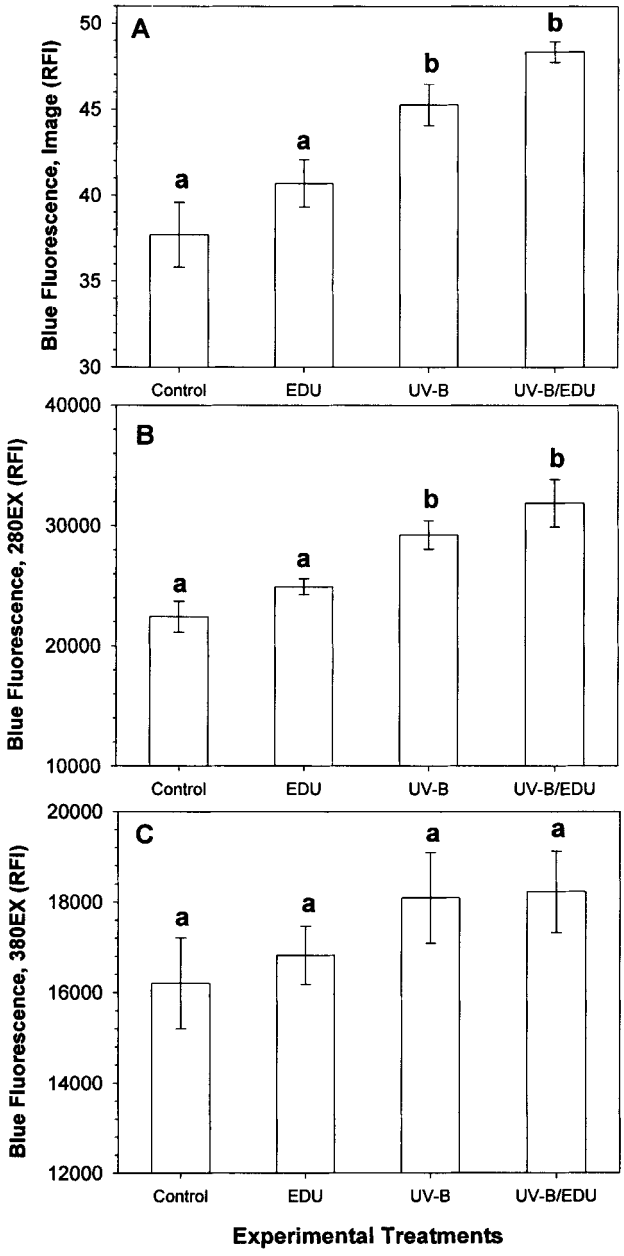

Figure 4. The relative blue (centered at 450 nm) fluorescence for the four experimental treatments (Control, EDU, UV-B, and combined UV-B/EDU) for the three measurement types: A) fluorescence image means (adaxial whole leaf surfaces), $F450_{FImage}$; B) fluorescence of leaf extracts, obtained from 280EX, $F450_{280EX}$; and C) fluorescence of leaf extracts, obtained from 380EX, $F450_{380EX}$. Error bars indicate ± SE and lower case letters indicate significantly different ($P \leq 0.05$) treatment means.

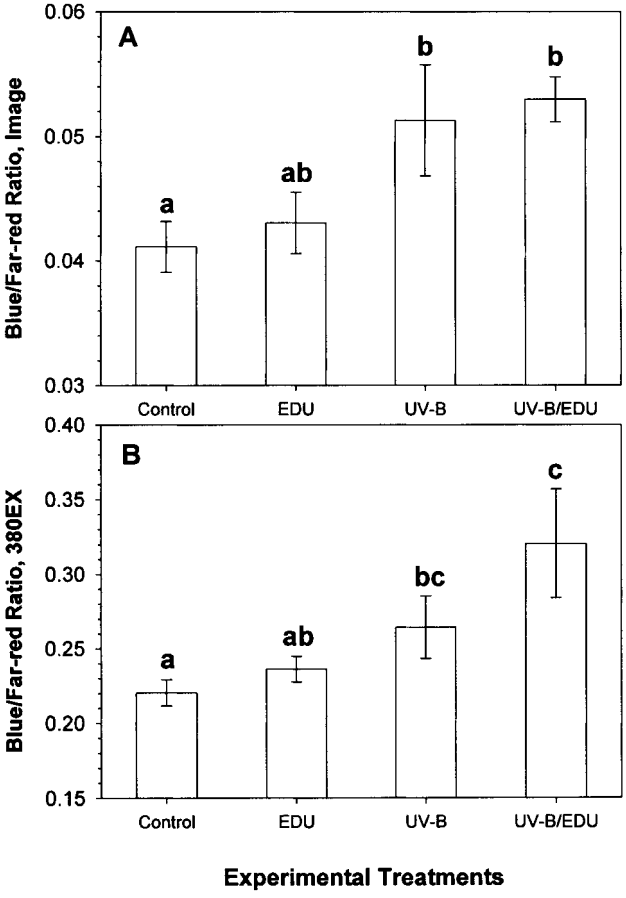

Figure 5. The blue/far-red fluorescence ratios for the four experimental treatments [Control, EDU, UV-B and combined UV-B/EDU] for: A) fluorescence ratio of images $F450/F740_{FImage}$ of adaxial whole leaf surfaces; and B) fluorescence ratio $F450/F730_{380EX}$ of extracts excited at 380 nm. Error bars indicate ± SE and lower case letters indicate significantly different ($P \leq 0.05$) treatments means.

agrees with results previously reported by Middleton et al. (1996) and Subhash et al. (1995). Several workers (Corp et al. 1997, Middleton et al. 1996) have suggested that this increase in the blue/far-red ratio may be caused by enhanced production of a blue fluorescing compound found to accumulate after UV-B induced degradation of rubisco. There is compelling evidence that the blue fluorescence band may be associated with certain cofactors of plant metabolism, pyridine and flavine nucleotides, e.g. water soluble reduced nicotinamide-adenine dinucleotide phosphate (NADPH), vitamin K_1, and xanthophyll (Cerovic et al. 1993, Chappelle et al. 1991). The prominence in the images of blue and green fluorescence in the veins (Fig. 2) may be due to the presence of plant phenolic compounds (e.g., hydroxy cinnamic acids, flavonols etc.) (Lang et al. 1994, Stober et al. 1994). The relatively higher red and far-red fluorescence observed in images of intercostal regions of the leaves is consistent with previous studies (Lichtenthaler 1996).

Many target sites of UV-B radiation have been reported (see e.g., Bornman et al. 1994, Tevini 1993 and references therein). The general consensus is that PS II is much more sensitive to UV-B radiation than is PS I (Middleton and Teramura 1994). Measurements of variable fluorescence as a measure of PS II activity, indicate that PS II activity is decreased by UV-B (Tevini et al. 1988). Under artificial or solar UV-B radiation, F_{max} is also decreased while F_o is increased (Tevini et al. 1988). UV-B treatment alone, or in combination with EDU, produced a significant reduction in the red/green fluorescence ratios of either images or 380EX emission spectra. The similar responses expressed by experimental treatments with the red/green fluorescence ratios obtained by either fluorescence imaging or by 380EX data support the validity of the imaging technique as a non-destructive diagnostic tool for assessing UV-B damage in plants. We suggest that the red/green ratio may be as useful an indicator of UV-B radiation stress as the previously identified blue/far-red (F450/F730) fluorescence ratio.

The agreement between the FImage and 280EX measurements in the blue and green spectra, not seen between FImage and 380EX, requires some additional explanation. Typically, RFI of 380EX for cucumber leaves in the blue and green wavelengths was 3–20 times lower than that in the red and far-red (Table 3). However, RFI from 280EX for F450 and F525 of cucumber leaves were 156 % and 374 % higher, respectively (Table 3), than comparable wavelengths of 380EX. The magnitude of all RFI from FImage exceeded those of 280EX and 380EX by a substantial amount (Table 3).

Another factor to be considered is the amount of biologically effective UV radiation that might have been emitted by

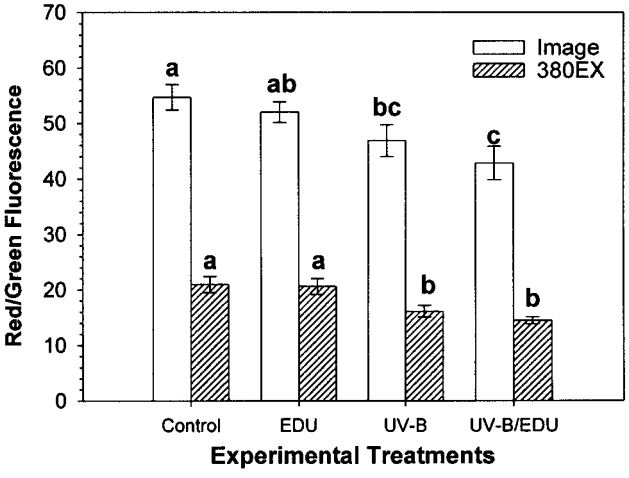

Figure 6. The red/green fluorescence ratios, $F680/F550_{FImage}$ and $F675/F525_{380EX}$, are shown for the four experimental treatments [Control, EDU, UV-B, and combined UV-B/EDU], where error bars indicate ± SE and lower case letters indicate significantly different ($P \leq 0.05$) treatment means.

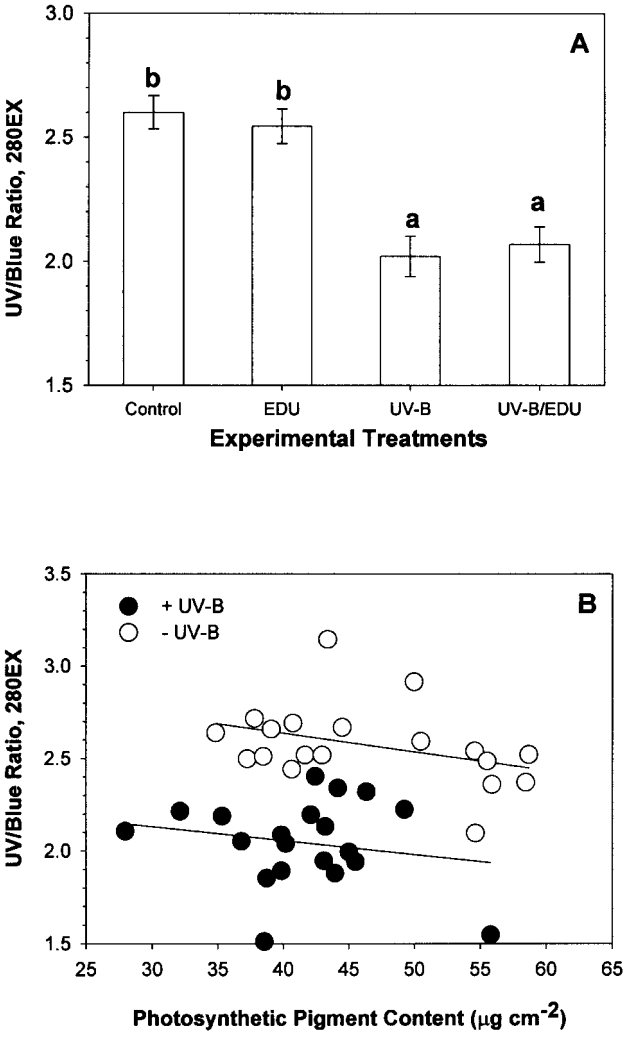

Figure 7. The UV/blue F315/F420$_{280EX}$ fluorescence ratio resulting from excitation of leaf extracts at 280 nm is shown: A) for the four experimental treatments [Control, EDU, UV-B, and combined UV-B/EDU], where error bars indicate ± SE and lower case letters indicate significantly different ($P \leq 0.05$) treatment means; and B) for the +UV and –UV treatments as a function of total photosynthetic pigment content (chlorophyll plus carotenoids, μg cm^{-2}), where $r^2 = 0.62$, with $P \leq 0.000$ for UV-B exposure and $P = 0.08$ for pigment content.

the 280 nm excitation source of the 280EX and the FIS; the latter emits a wide excitation band (300–400 nm) that includes UV-B$_{BE}$ which diminishes between 300–320 nm. At this time, spectroradiometric measurements have been made of the 280EX source but not of the FIS source. The 280 nm source was found to emit 320.6 mW m^{-2} of biologically effective UV radiation from 260–320 nm. As might be expected, the amount of UV-B$_{BE}$ emitted by the 380EX source was negligible. It would seem likely that the relatively highly energetic blue and green emissions captured by FImage, and most associated with leaf veins, were stimulated by the UV-B and UV-A excitation wavelengths that penetrated the epidermis.

In contrast, the red and far-red emissions (indicative of chlorophyll) captured by FImage in the interveinal regions were most likely associated with the epidermal layer and upper mesophyll tissue, but not the full tissue profile, due to the lower resonance energy at these longer wavelengths. Therefore, 380EX RFI for the extracts which included compounds from the full tissue profile might be expected to differ from images in the longer wavelengths, as observed. However, the UV-B induced reduction in chlorophyll that we obtained was not reflected in the far-red/red fluorescence ratios in either the 380EX measurement of extracts or the images of whole leaves, even though several reports have indicated that this ratio is a useful stress indicator of *in vivo* chlorophyll content (D'Ambrosio et al. 1992, Hak et al. 1990, Lichtenthaler et al. 1990).

Our results are consistent with those of Subhash et al. (1995), who also found no significant change in the related F685/F730 ratio following UV treatment of *Salvia splendens*. In our current study, photosynthetic pigment content was, instead, correlated with the UV/blue (F315/F420) ratio of 280EX measurements. UV-B altered this pigment vs. UV/blue relationship (Fig. 7 B), producing relatively lower emissions at wavelengths associated with protein fluorescence (315 nm) compared with blue-fluorescing compounds at 420 nm. These results suggest that UV-B exposure caused a shift in the relative production or maintenance of leaf compounds associated with the synthesis or recycling of chlorophyll.

The decrease in chlorophyll *a/b* ratio when cucumber plants were irradiated with UV-B radiation is consistent with results obtained for *Brassica napus* L. by Larsson et al. (1998). They suggested that this may have been due to a faster breakdown or decreased synthesis of chlorophyll *a* as compared to chlorophyll *b,* although chlorophyll *b* also decreased. Although UV-B radiation often has a negative effect on chlorophyll concentration (Strid and Porra 1992), Larsson et al. (1998) observed no effect of UV-B radiation on chlorophyll *a* or chlorophyll *b* concentration. In our study, we observed a decrease in chlorophyll *a* concentration under UV-B irradiation but no effect on chlorophyll *b* concentration. The combined UV-B/EDU treatment, however, significantly reduced the concentration of chlorophyll *a,* chlorophyll *b,* total photosynthetic pigments, and the chlorophyll/carotenoid ratio.

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References

Adamse P, Britz SJ (1992) Amelioration of UV-B damage under high irradiance. I. Role of photosynthesis. Photochem Photobiol 56: 645–650

Agrawal M, Agrawal SB, Krizek DT, Kramer GF, Lee EH, Mirecki RM, Rowland RA (1991) Physiological and morphological responses of snapbean plants to ozone stress as influenced by pretreatment with UV-B radiation. In: Abrol YP et al (eds) Impact of Global Climate Changes on Photosynthesis and Plant Productivity. Oxford & IBH Publ Co, New Delhi, India, pp 133–146

Bennett JH, Lee EH, Heggestad HE, Olsen RA, Brown JC (1981) Ozone injury and aging in leaves: Protection by EDU. In: Rogers MAJ, Powers EL (eds) Oxygen and Oxy-Radicals in Chemistry and Biology. Academic Press, New York, pp 604–605

Blumthaler M, Ambach W (1988) Solar UV-B albedo of various surfaces. Photochem Photobiol 48: 85–88

Bornman JF, Sundby-Emanuelsson C, Cen Y-P, Alenius C (1994) UV-B radiation and the photosynthetic process. In: Biggs RH, Joyner MEB (eds) Stratospheric Ozone Depletion/UV-B Radiation in the Biosphere. Springer-Verlag, Berlin, pp 345–348

Caldwell MM (1971) Solar UV irradiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology, Vol 6. Academic Press, New York, NY, pp 131–177

Carnahan JE, Jenner EL, Wat EKW (1978) Prevention of ozone injury to plants by a new protectant chemical. Phytopathology 68: 1225–1229

Cathey HM, Heggestad HE (1982) Ozone sensitivity of herbaceous plants: Modification by ethylenediurea. J Am Soc Hort Sci 107: 1035–1042

Cerovic ZG, Bergher M, Goulas Y, Tosti S, Moya I (1993) Simultaneous measurement of changes in red and blue fluorescence in illuminated isolated chloroplasts and leaf pieces: The contribution of NADPH to the blue fluorescence signal. Photosynth Res 36: 103–204

Chappelle EW, Kim MS, McMurtrey JE (1992) Ratio analysis of reflectance spectra (RARS): An algorithm for the remote estimation of the concentrations of chlorophyll *a,* chlorophyll *b,* and carotenoids in soybean leaves. Remote Sens Environ 39: 239–247

Chappelle EW, McMurtrey JE, Kim MS (1991) Identification of the pigment responsible for the blue fluorescence band in the laser induced fluorescence (LIF) spectra of green plants, the potential use of this band in remotely estimating rates of photosynthesis. Remote Sens Environ 36: 213–218

Corp LA, McMurtrey JE, Chappelle EW, Daughtry CST, Kim MS (1997) UV band fluorescence (*in vivo*) and its implications for the remote assessment of nitrogen supply in vegetation. Remote Sens Environ 61: 110–117

Corp LA, McMurtrey JE, Chappelle EW, Kim MS, Daughtry CST (1996) Optimizing fluorescence excitation wavelengths for the detection of stress in vegetation. Proc Internat Geoscience and Remote Sensing Symp 3: 1812–1815

D'Ambrosio N, Szabo K, Lichtenthaler HK (1992) Increase of the chlorophyll fluorescence ratio F690/F735 during the autumnal chlorophyll breakdown. Radiat Environ Biophys 31: 51–62

Hak R, Lichtenthaler HK, Rinderle U (1990) Decrease of the chlorophyll fluorescence ratio F690/F730 during greening and development of leaves. Radiat Environ Biophys 29: 329–336

Herman JR, Bhartia PK, Ziemke J, Ahmad Z, Larko D (1996) UV-B increases (1979–1992) from decreases in total ozone. Geophys Res Lett 23: 2117–2120

Kim MS, Krizek DT, Daughtry CST, McMurtrey JE, Sandhu R, Chappelle EW, Corp LA, Middleton EM (1997) Fluorescence imaging system: Application for the assessment of vegetation stresses. SPIE EUROPT Series 2959: 4–13

Kosta-Rick R, Manning WJ (1993) Dose-response studies with the antiozonant ethylenediurea (EDU), applied as a soil drench to two growth substrates, on greenhouse-grown varieties of *Phaseolus vulgaris* L. Environ Pollut 82: 63–72

Krizek DT (1978) Differential sensitivity of two cultivars of cucumber (*Cucumis sativus* L.) to increased UV-B irradiance. I. Dose-response studies. Final Report on Biological and Climatic Effects Research, USDA-EPA, Washington, DC, 1–33

Krizek DT, Mirecki RM, Kramer GF (1994) Growth analysis of UV-B irradiated cucumber seedlings as influenced by photosynthetic photon flux source and cultivar. Physiol Plant 90: 593–599

Lang M, Lichtenthaler HK, Sowinska M, Heisel F, Miehe JA (1996) Fluorescence imaging of water and temperature stress in plant leaves. J Plant Physiol 148: 613–621

Lang M, Lichtenthaler HK, Sowinska M, Heisel F (1994) Blue, green, and red fluorescence signatures and images of tobacco leaves. Bot Acta 107: 230–236

Lang M, Stober F, Lichtenthaler HK (1991) Fluorescence emission spectra of plant leaves and plant constituents. Radiat Environ Biophys 30: 333–347

Larsson EH, Bornman JF, Asp H (1998) Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. J Exp Bot 49: 1031–1039

Lee EH, Bennett JA (1985) Superoxide dismutase: A possible protective enzyme against ozone injury in snapbeans (*Phaseolus vulgaris* L.). Physiol Plant 76: 479–484

Lee EH, Kramer GF, Rowland RA, Agrawal M (1992) Antioxidants and growth regulators counter the effects of O_2 and SO_2 in crop plants. Agri Ecosystem and Environ, 38: 99–106

Lichtenthaler HK (1990) Applications of chlorophyll fluorescence in stress physiology and remote sensing. In: Steven M, Clark JA (eds) Applications of Remote Sensing in Agriculture. Butterworths Scientific Ltd, London, pp 287–305

Lichtenthaler HK (1996) Vegetation stress: an introduction to the stress concept in plants. J Plant Physiol 148: 4–14

Lichtenthaler HK (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends Plant Sci 2 (8): 316–320

Lichtenthaler HK, Hak R, Rinderle U (1990) The chlorophyll fluorescence ratio F690/F730 in leaves of different chlorophyll content. Photosynth Res 25: 295–298

Lichtenthaler HK, Rinderle U (1988) The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry, 19 (Suppl. 1): 29–85

Middleton EM, Chappelle EW, Cannon TA, Adamse P, Britz SJ (1996) Initial assessment of physiological response to UV-B irradiation using fluorescence measurements. J Plant Physiol 148: 69–77

Middleton EM, Teramura AH (1994) Understanding photosynthesis, pigment and growth responses induced by UV-B and UV-A irradiances. Photochem Photobiol 60: 38–45

Miller JE, Pursley WA, Heagle AS (1994) Effects of ethylenediurea on snap bean at a range of ozone concentrations. J Environ Qual 23: 1082–1089

Mirecki RM, Teramura AH (1984) Effects of ultraviolet-B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 74: 475–480

Sandhu RK, Kim MS, Krizek DT, Middleton EM (1997) Fluorescence imaging and chlorophyll fluorescence to evaluate the role of EDU in UV-B protection in cucumber. Proceedings SPIE Aerosense '97, SPIE Vol 3059, pp 42–50

Silvius JE, Kramer DF, Lee DR (1978) Carbon assimilation and translocation in soybean leaves at different stages of development. Plant Physiol 62: 54–58

Snell JFH, Van Kooten O (eds) (1990) The use of chlorophyll fluorescence and other non-invasive spectroscopic techniques in plant stress physiology. Photosynth Res (Spec Issue) 25: 146–332

Stober F, Lang M, Lichtenthaler HK (1994) Blue, green and red fluorescence emission signatures of green, etiolated, and white leaves. Remote Sens Environ 47: 65–71

Strid A, Chow WS, Anderson JM (1990) Effects of supplementary ultraviolet-B radiation on photosynthesis in *Pisum sativum*. Biochem Biophys Acta 1020: 260–268

Strid A, Porra RJ (1992) Alterations in pigment content in leaves of *Pisum sativum* after exposure to supplementary UV-B. Plant Cell Physiol 33: 1015–1023

Subhash N, Mazzinghi P, Agati G, Fusi F, Lercari B (1995) Analysis of laser-induced fluorescence line shape of intact leaves: application to UV stress detection. Photochem Photobiol 62: 711–718

Tevini M (ed) (1993) UV-B Radiation and Ozone Depletion: Effects on Humans, Animals, Plants, Microorganisms, and Material. Lewis Publ, Boca Raton, FL, 248 pp

Tevini M (1994) Physiological changes in plants related to UV-B radiation: An overview. In: Biggs RH, Joiner MEB (eds) Stratospheric Ozone Depletion/ UV-B Radiation in the Biosphere. NATO ASI Series I, Vol 18, Springer-Verlag, Berlin, Heidelberg, pp 37–56

Tevini M, Grusemann P, Fieser G (1988) Assessment of UV-B stress by chlorophyll fluorescence analysis. In: Lichtenthaler HK (ed) Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology and Remote Sensing. Kluwer Academic Publishers, Dordrecht, pp 229–238

Tevini M, Pfister K (1985) Inhibition of Photosystem II by UV-B radiation. Z Naturforsch 40 c: 129–133